Response to Reviewer1 and Reviewer2

We wish to express our appreciation to the two reviewers for their insightful comments on our paper. The comments have helped us significantly improve the paper. We described all changes highlighted in red color.

Reviewer1

Comment1: This manuscript by Eto and colleagues described results of a study which demonstrate that Japanese males aged≤ 49 years carrying TT genotype on *GNB3* C825T might have significantly higher risk in relation to SBP elevation of ≥130 mmHg. This is an appealing study which emphasize that *GNB3* C825T polymorphism may be a useful genetic marker for hypertension. The amount and quality of work is good enough to make the manuscript publishable but I have some queries which are as follows:

Response1: We are grateful for your carefully reviewing the manuscript and making valuable comments.

Commnet2: * On page 4, Abstract part: AIME must be replaced by AIM.

Response1: Thank you for your comment. According to your comment, the AIM in the Abstract was replaced by the AIM in the text.

"AIM: To investigate whether *GNB3* C825T single nucleotide polymorphism (SNP) contributes to systolic blood pressure (SBP) ≥ 130 mmHg in a large-scale cross-sectional study among the Japanese population with diastolic blood pressure (DBP) < 85 mmHg. "

Commnet3: * Abstract part: SBP should be provided with the full name when it appears for the first time, even though it is well known to specialist.

Response2: Thank you for your comments. We have changed from SBP and DBP to systolic blood pressure (SBP) and diastolic blood pressure (DBP), respectively, at the first appearance in the Abstract.

"… contributes to systolic blood pressure (SBP) ≥ 130 mmHg in a large-scale cross-sectional study among the Japanese population with diastolic blood pressure (DBP) < 85 mmHg."

Comment4: * The introduction section is too large and described too much of the well known GPCRs activation.

Response4: Thank you for your comments. According to the comments, the related part of the Introduction section was revised as the following by deleting the redundant descriptions about the GPCRs activation. It could make about 55% reduction of the volume (from 701 words to 328 words).

"… Since the JNC-7 proposal, numerous studies including Multiple Risk Factor Intervention Trial (MRFIT), the Framingham Heart Study, and the TRial of Preventing Hypertension (TROPHY) study have investigated the risk of pre-HT for various types of adverse outcomes. As a result, different effects of two BP ranges in pre-HT patients on future outcomes have been demonstrated [5,6,9-11]. Per the guidelines provided by the Japanese Society of Hypertension (JSH) in 2009, the target level of BP control was less than 130/85 mmHg in young and middle-aged individuals. Nowadays, therapeutic lifestyle interventions such as weight loss, salt restriction, and exercise are recommended, especially at SBP levels ≥ 130 mmHg to prevent cardiovascular disease and its progression to HT, although drug intervention is recommended at SBP ≥ 140 mmHg. HT is a multi-factorial disorder caused by the interaction between genetic and environmental factors. Many studies have sought to identify genetic variants linked to HT, but there have been few studies to correlate genetic variants with isolated SBP elevation ≥130 mmHg.

Heterotrimeric G-proteins, which consist of three subunits (α u β and γ), are located on the cytoplasmic side of the cell membrane and relay signals from G protein-coupled receptors (GPCRs) to downstream effectors [12, 13)]. In humans, there are at least 16, 5, and 12 different genes encoding α od β , and g 12 different genes he [15-18]. The combinations of the three subunits affect the function of G-proteins. The *GNB3* gene is one of the five genes encoding the G-protein e of the five genes the C825T SNP within exon 10 of *GNB3* does not change the encoded amino acid (ser275ser), the 825T allele expresses a truncated splice variant of G β 3, termed G β 3s, which contains 41 fewer amino acids. The missing domain is the functionally important region that forms tight binding with hich in-coupled receptors (GPCRs) to downstream

effect original transduction by forming a functional heterodimer with G γ 5 [14, 17, 19, 20].”

Comment5: * In discussion part, the authors should provide more detailed discussion to compare the difference of this study with other reported studies of *GNB3* C825T polymorphism in Japanese population, rather than a brief citation like “45.7-53.1% in Japanese[2, 24, 28, 35]”.

Response5: Thank you for the valuable comments. According to the comments, the description following concerning the comparison of the difference of this study with other reported studies of *GNB3* C825T polymorphism in Japanese population was inserted into the Discussion.

“There have been no reports regarding the associations between *GNB3* C825T and the risk of SBP ≥ 130 mmHg in a Japanese population. This is the first study demonstrating a significant association between *GNB3* C825T and the risk of SBP ≥ 130 mmHg in young Japanese males. There have been several studies regarding the associations between *GNB3* C825T and HT in Japanese populations [24,25,26,28, 30]. In these studies, hypertensive cases were selected as SBP ≥ 160 and/or DBP ≥ 95 mmHg[26,28], SBP ≥ 140 and/or DBP ≥ 90 mmHg [24,25, 30], or SBP ≥ 134 and/or DBP ≥ 79 mmHg by ambulatory blood pressure monitoring (ABPM) [26]. The subjects taking anti-hypertensive medication were included as cases. Normotensive controls were defined as SBP < 140 and DBP < 90 mmHg[24,25,26,28, 30], except in one study in which the criteria of controls were SBP < 134 and DBP < 79 mmHg by ABPM [26]. Although Izawa, *et al*. [25] demonstrated a significant association between *GNB3* C825T and HT only in males (mean age 56.3 years), the *GNB3* genotype distribution did not significantly differ between cases and controls in the analyses of all subjects. In this study, newly diagnosed cases were defined as SBP higher than or equal to 130 mmHg and DBP less than 85 mmHg. In addition, subjects taking anti-hypertensive medication at the time of the study or with a history of HT were excluded. Therefore, the different definitions of hypertension might affect the conclusions about the relationship between *GNB3* and HT. The findings in this study were obtained by using a large sample sized genome banking data collected from all over Japan. These results suggest that the *GNB3* C825T SNP is a likely risk factor for pre-HT in Japanese young males.”

Comment6: * Why the authors choose the age of 49 as the standard for grouping the population, is it reasonable? How the authors eliminate the influence of aging on the analysis.

Response6: Thank you for your comment. The sentences following has inserted into the Data analysis;

"The prevalence of cases defined as SBP ≥ 130 and DBP < 85 mmHg was different between subjects ≤49 and ≥50 years in both males and females. Thus, statistical analyses were performed in four subgroups (males ≤ 49 years, males ≥ 50 years, females ≤ 49 years, and females ≥ 50 years), respectively. The genotype distributions within each subgroup were consistent with Hardy-Weinberg equilibrium."

Reviewers2

Comment1: The role of G-protein activation in cardiovascular disorders is well-known. G-Protein β3 Subunit Gene (*GNB3*) C825T polymorphism is associated with increased intracellular signal transduction. However, the relationship between the *GNB3* gene polymorphism (C825T) and blood pressures is inconsistent in different populations. This paper reports a study designed to examine the association between the *GNB3* gene polymorphism (C825T) and SBP elevation of ≧130 mmHg in a cross-sectional study of Japanese. Unfortunately, this paper is of poor quality, and the study was not necessarily very well designed. It is not suitable for publication in its present form.

Response1: We are grateful for your carefully reviewing the manuscript and making valuable comments. The initial manuscript has been revised appropriately on the basis of the reviewer's comment. Therefore, we believe that the revised manuscript has been significantly improved as suitable for the publication on the journal.

Comment2: Abstract – The authors state clearly the main results and conclusions of this paper.

Response2: Thank you for your comment. We changed the sentence of them to the description following;

"This study indicates that the TT genotype of the *GNB3* C825T SNP may contribute to

SBP elevation of greater than 130 mmHg compared to the CC genotype in Japanese males aged ≤49 years."

Comment3: Introduction – this section is too long. Do not review the subject extensively. The rationale of the study is not sufficiently explained. The authors need to make a more convincing argument of why one would want to study the relationship *GNB3* gene polymorphism (C825T) and SBP elevation of ≧130 mmHg in Japanese. I am unconvinced after the introduction that this is an important gap in knowledge.

Response3: Thank you for your comments. According to the comments, the related part of the Introduction section was revised as the following by deleting he redundant descriptions. It could make about 60% reduction of the volume (from 701 words to 309 words).

"… Since the JNC-7 proposal, numerous studies including Multiple Risk Factor Intervention Trial (MRFIT), the Framingham Heart Study, and the TRial of Preventing Hypertension (TROPHY) study have investigated the risk of pre-HT for various types of adverse outcomes. As a result, different effects of two BP ranges in pre-HT patients on future outcomes have been demonstrated [5,6,9-11]. Per the guidelines provided by the Japanese Society of Hypertension (JSH) in 2009, the target level of BP control was less than 130/85 mmHg in young and middle-aged individuals. Nowadays, therapeutic lifestyle interventions such as weight loss, salt restriction, and exercise are recommended, especially at SBP levels ≥ 130 mmHg to prevent cardiovascular disease and its progression to HT, although drug intervention is recommended at SBP ≥ 140 mmHg. HT is a multi-factorial disorder caused by the interaction between genetic and environmental factors. Many studies have sought to identify genetic variants linked to HT, but there have been few studies to correlate genetic variants with isolated SBP elevation ≥130 mmHg.

Heterotrimeric G-proteins, which consist of three subunits (α u β and γ), are located on the cytoplasmic side of the cell membrane and relay signals from G protein-coupled receptors (GPCRs) to downstream effectors [12, 13)]. In humans, there are at least 16, 5, and 12 different genes encoding α od β , and g 12 different genes he [15-18]. The combinations of the three subunits affect the function of G-proteins. The *GNB3* gene is one of the five genes encoding the G-protein e of the five genes the

C825T SNP within exon 10 of *GNB3* does not change the encoded amino acid (ser275ser), the 825T allele expresses a truncated splice variant of G β 3, termed G β 3s, which contains 41 fewer amino acids. The missing domain is the functionally important region that forms tight binding with hich in-coupled receptors (GPCRs) to downstream effect original transduction by forming a functional heterodimer with G γ 5 [14, 17, 19, 20].”

Comment4: Methods – Why stratified the subjects by age (≤49 years and ≥50 years)? This needs to be stated.

Response4: Thank you for your comment. The sentences following has inserted into the Data analysis;

“The prevalence of cases defined as SBP ≥ 130 and DBP < 85 mmHg was different between subjects ≤49 and ≥50 years in both males and females. Thus, statistical analyses were performed in four subgroups (males ≤ 49 years, males ≥ 50 years, females ≤ 49 years, and females ≥ 50 years), respectively. The genotype distributions within each subgroup were consistent with Hardy-Weinberg equilibrium.”

Comment5: Results – In the multivariable logistic regression analysis, the ORs was only adjusted for age and BMI (without other potential confounding factors?). Therefore, the association found in this paper must still be regarded as tentative. Discussion – The limitations of this study should be discussed.

Response5: Thank you for your comments. This issues were the study limitations, therefore, the description following have been added into the study limitation section;

“Some limitations need to be noted when considering the results from this study. First, logistic regression analysis was adjusted only for age and BMI as covariates. We need to reassess the interactions in combination with other potential confounding factors (such as environmental factors). Second, the cross-sectional design does not allow us to make any conclusive statements. A significant association between *GNB3* C825T and isolated SBP elevation ≥130 mmHg in males aged ≤49 years needs to be reassessed in a prospective cohort study.”

Comment6: Reference – 1) only 3 of 38 are the last 5 years. 2) The references are in variable format and need to be consistent and in the format required by the Journal.

Response6: Thank you for your comment. The format of the references was consistent based on the format required by the Journal.